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      (FILE 'HOME' ENTERED AT 15:46:21 ON 12 NOV 2003)

      FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, USPATFULL' ENTERED AT 15:46:37
      ON 12 NOV 2003
L1          4512 S (ERYTHROC? OR (RED CELL?)) (5A) MATUR?
L2           244 S L1 (6P) FLOW CYTOMET?
L3           486 S L1 (6P) ERYTHROBLAST?
L4           736 S L1 (6P) (ERYTHROBLAST? OR NUCLEATED)
L5            82 S L4 AND L2
L6            52 DUP REM L5 (30 DUPLICATES REMOVED)
L7             9 S L6 AND ((NUCLEOTIDE OR NUCLEAR) (2A) STAIN?)

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=> s l6 and ((nucleotide or nuclear) (2a) stain?)
L7             9 L6 AND ((NUCLEOTIDE OR NUCLEAR) (2A) STAIN?)

=> d l7 ibib ab kwic 1-9

L7   ANSWER 1 OF 9  USPATFULL on STN
ACCESSION NUMBER:        2003:43481  USPATFULL
TITLE:                   Consumable tube for use with a flow cytometry-based
                         hematology system
INVENTOR(S):             Roche, John W., Scarborough, ME, UNITED STATES
                         Hansen, W. Peter, Canaan, NY, UNITED STATES
                         Julian, Marcus F., Medford, MA, UNITED STATES
                         Flynn, Harold C., JR., Scarborough, ME, UNITED STATES
                         Russell, James W., North Yarmouth, ME, UNITED STATES
                         Coleman, Michelle L., Yarmouth, MI, UNITED STATES
                         Crews, Harold R., Coral Springs, FL, UNITED STATES

                              NUMBER         KIND    DATE
                         ---------------- ------ --------
PATENT INFORMATION:      US 2003030783      A1   20030213
APPLICATION INFO.:       US 2002-159944     A1   20020531  (10)
RELATED APPLN. INFO.:    Division of Ser. No. US 2000-715593, filed on 17 Nov
                         2000, PENDING

                                NUMBER         DATE
                         ------------------ --------
PRIORITY INFORMATION:    US 2000-208849P    20000602 (60)
DOCUMENT TYPE:           Utility
FILE SEGMENT:            APPLICATION
LEGAL REPRESENTATIVE:    LYON & LYON LLP, 633 WEST FIFTH STREET, SUITE 4700, LOS
                         ANGELES, CA, 90071
NUMBER OF CLAIMS:        73
EXEMPLARY CLAIM:         1
NUMBER OF DRAWINGS:      29 Drawing Page(s)
LINE COUNT:              2848
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB       The present invention is a flow cytometry-based hematology system useful
         in the analysis of biological samples, particularly whole blood or
         blood-derived samples. The system is capable of determining at least a
         complete blood count (CBC), a five-part white blood cell differential,
         and a reticulocyte count from a whole blood sample. The system
         preferably uses a laser diode that emits a thin beam to illuminate cells
         in a flow cell and a lensless optical detection system to measure one or
         more of axial light loss, low-angle forward scattered light, high-angle
         forward scattered light, right angle scattered light, and time-of-flight
         measurements produced by the cells. The lensless optical detection
         system contains no optical components, other than photoreactive
         elements, and does not include any moving parts. Finally, the system
         uses a unique system of consumable reagent tubes that act as reaction
         chambers, mixing chambers, and waste chambers for the blood sample
         analyses. The consumable tubes incorporate reference particles, which
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act as internal standards to ensure that the dilutions made during processing of the samples have been carried out correctly, and to ensure that the instrument is working properly. The present invention also relates to methods for using the system.

SUMM [0002] This invention relates in general to bioparticle analysis, and more specifically to **flow-cytometry**-based methods and devices for performing automated blood cell analysis.

SUMM . . . liter. RBCs are responsible for oxygen and carbon dioxide transport within the circulatory system. In many mammals, including humans, normal **mature red cells** have a bi-concave cross-sectional shape and lack nuclei. RBCs can range in diameter between 4 and 9 microns, depending on. . .

SUMM . . . slightly immature RBC is referred to as a reticulocyte, and the very immature forms of RBCs are broadly classified as **nucleated** red blood cells (NRBCs). Higher level non-mammalian animals, such as birds, reptiles, and amphibians, have exclusively **nucleated** RBCs in their blood.

SUMM . . . this application, the terms "white blood cells", "white cells", "leukocytes", and "WBCs" are used interchangeably to refer to the non-hemoglobin-containing **nucleated** blood cells present in the circulation as described above.

SUMM . . . method for counting nucleated red blood cells (NRBCs) by first lysing red blood cells, then exposing the NRBCs to a **nuclear stain** and measuring fluorescence and light scatter. U.S. Pat. No. 5,733,784 (Studholme et al.) teaches a method for measuring the reticulocyte. . .

L7 ANSWER 2 OF 9 USPATFULL on STN

ACCESSION NUMBER:	2001:33090 USPATFULL
TITLE:	Method for enumerating blood cells
INVENTOR(S):	Deka, Chiranjit, Miami, FL, United States Wyatt, James L., Plantation, FL, United States Gordon, Kristie M., Coral Gables, FL, United States
PATENT ASSIGNEE(S):	Coulter International Corp., Miami, FL, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6197593	B1	20010306	
APPLICATION INFO.:	US 1998-175494		19981020	(9)
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	Granted			
PRIMARY EXAMINER:	Wallenhorst, Maureen M.			
LEGAL REPRESENTATIVE:	Bak, Mary E., Alter, Mitchell E.			
NUMBER OF CLAIMS:	18			
EXEMPLARY CLAIM:	1			
NUMBER OF DRAWINGS:	18 Drawing Figure(s); 9 Drawing Page(s)			
LINE COUNT:	811			

AB A method for enumerating and distinguishing between blood cell populations in a biological sample includes the steps of contacting a biological sample with a cell membrane-permeant, red-excited, nucleic acid binding dye, without significantly disrupting the integrity of the cells; exciting this sample with light in one red wavelength; and measuring fluorescence emitted from different cell populations in the sample. This method is particularly desirable for enumerating different WBC subpopulations using flow cytometry. This method is also useful for enumerating reticulocytes or NRBC or mature RBC. This method is enhanced by pretreating the sample with a nucleic acid-specific blocking agent. Dyes useful in this method include, without limitation, SYTO.RTM.17 dye, SYTO.RTM.159 dye, SYTO.RTM.60 dye, SYTO.RTM.61 dye, SYTO.RTM.62 dye, SYTO.RTM.63 dye, and SYTO.RTM.64 dye.

SUMM . . . fluorescence in free solution, both oxazine 720 and oxazine 750 have been used as gain media in dye lasers. For **flow cytometric** analysis of cells, however, bright fluorescence of

unbound molecules is a highly undesirable attribute for dyes used to stain specific. . .

SUMM . . . in excitation and emission spectra. Eukaryotic cells incubated with SYTO.RTM. dyes reportedly show cytoplasmic or mitochondrial staining as well as **nuclear staining** (Molecular Probes product insert). These dyes have also been reported to produce minimal background fluorescence from unbound dye and enhancement. . .

SUMM Thus, in one aspect, the present invention provides an improved method for differentiating between leukocyte populations by fluorescence **flow cytometry**. More specifically, in one embodiment, the method permits enumerating and distinguishing between different white blood cell (WBC) subpopulations in a. . .

SUMM In another embodiment, this method permits the differentiation of reticulocytes from **mature red cells** by fluorescence **flow cytometry** based on nucleic acid staining and fluorescence measurement at one wavelength and with high specificity for nucleic acid. This method. . .

SUMM In still another aspect, the invention provides a method for differentiating reticulocytes, which contain RNA, or **nucleated** red blood cells (NRBC), that contain DNA, from mature red blood cells that contain neither DNA nor RNA, by fluorescence **flow cytometry** comprising contacting a biological sample containing reticulocytes NRBC and mature RBC and optionally other cell populations, as described above, with. . .

DRWD . . . red cells stained with SYTO.RTM.62 stain according to the present method. The rectangular gate is designed to distinguish between non-fluorescent **mature red cells** and fluorescent reticulocytes. Reticulocytes are resolved from the **mature red cells** by the high fluorescence signal from the RNA bound SYTO.RTM.62 stain. This figure shows results for a sample having low. . .

DRWD . . . the CPO staining method described in U.S. Pat. No. 5,639,666. Measurements for CPO method were conducted in a standard XL.TM. **flow cytometer** using a 488 nm argon laser as the excitation source. See Example 3 below.

DETD . . . one minute in duration for red cells mediated by formic acid [Coulter Corp.] for preparing WBCs for analysis in XL **flow cytometer**, and analyzed.

DETD . . . U.S. Pat. No. 3,962,125) and incubating the mixture for approximately 1 minute. Thereafter the cells were analyzed in an XL **flow cytometer** equipped with a red HeNe laser, with approximately 11.5 mW power incident on the beam shaping optics. Forward light scatter. . .

DETD . . . shown in FIG. 6E, for example. Thus, reticulocytes stained with SYTO.RTM.62 stain using the present method are resolved from the **mature red cells** by the high fluorescence signal from the RNA bound SYTO.RTM.62 stain.

DETD . . . cells in a forward scatter vs. fluorescence dotplot, shown for example in FIG. 6C, was consistent with the distribution of **mature red cells** and reticulocytes previously shown by Tanke et al., cited above, in relation to their work on Pyronin Y based reticulocyte. . .

DETD . . . according to the description of U.S. Pat. No. 5,639,666. Measurements for the CPO method were conducted in a standard XL **flow cytometer** using a 488 nm argon laser as the excitation (illumination) source. Forward scatter, side scatter and two fluorescence parameters were. . . bound CPO). The red cells were gated on SS vs. FS dotplot. An automated gating algorithm, available in commercial XL **flow cytometers**, calculated reticulocyte percentage from a DNA vs. RNA fluorescence dotplot.

DETD . . . according to the present method and the results obtained by independent measurements based on CPO fluorescence in a standard XL **flow cytometer** are correlated in FIG. 7, in which the percentage of the fluorescent subpopulation corresponded closely to

reticulocyte percentage for each. . .

CLM What is claimed is:

5. The method according to claim 4 wherein said RBC are selected from the group consisting of **mature red cells**, reticulocytes and NRBC.

8. The method according to claim 1 wherein said sample is excited and fluorescence is measured by a **flow cytometer**.

L7 ANSWER 3 OF 9 USPATFULL on STN

ACCESSION NUMBER: 1999:96277 USPATFULL

TITLE: Method and apparatus for performing automated analysis

INVENTOR(S): Chupp, Vernon L., Los Altos, CA, United States
Lobban, Peter E., Palo Alto, CA, United States
Kim, Young Ran, Sunnyvale, CA, United States
Larue, Roderick Walton, Sebastopol, CA, United States
Stuart, John Paul, Mt. View, CA, United States

PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5939326		19990817
APPLICATION INFO.:	US 1997-816712		19970313 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1995-482678, filed on 7 Jun 1995, now patented, Pat. No. US 5656499 which is a continuation-in-part of Ser. No. US 1994-283379, filed on 1 Aug 1994, now abandoned

DOCUMENT TYPE: Utility

FILE SEGMENT: Granted

PRIMARY EXAMINER: Le, Long V.

LEGAL REPRESENTATIVE: Bach, Mark C.

NUMBER OF CLAIMS: 8

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 111 Drawing Figure(s); 61 Drawing Page(s)

LINE COUNT: 4301

AB A device for analyzing a whole blood sample is provided. The device comprises a conventionl hematology analyzer integrated with a fluorescence cyometry analyzer. A controller is provided for controlling the analyzers, obtaining and utilizing data from both and reporting a quantitative result. Methods are also provided for analyzing a whole blood sample. One such method comprises the steps of performing on a single instrument an analysis of impedance associated with the blood sample, an analysis of light scatter associated with the blood sample, and an analysis of fluorescence associated with the blood sample. Data is collected and utilized. A result is reported.

SUMM . . . relates to methods and devices for performing automated blood cell analysis by integrating "impedance," "light scattering," and "fluorescence" analysis and **flow cytometric** techniques. This invention also relates to a multipurpose reagent system and a method for rapid analysis of a whole blood. . .

SUMM The blood in an adult usually contains about 4.5 to 5 million RBCs or **erythrocytes** per cubic millimeter. **Mature** RBCs have no nuclei and are generally shaped as circular biconcave disks with a diameter of about 7.5 to 8. . .

SUMM Peripheral blood also contains **red cells** of earlier **maturation** levels which are important diagnostic indicators. Two of these are reticuldcytes and **nucleated** red blood cells.

SUMM At the earliest stage of development the red cell consists mostly of nucleus, and is referred to as an **erythroblast**. As the **erythroblast** matures, the nucleus becomes smaller, anucleolate, and more nearly spherical. Subsequent maturity involves a complete loss of nucleus. The immature red cells that retain a nucleus are referred to

as **nucleated** red blood cells (NRBCs). The NRBC count has been useful in patient monitoring under many disease states. However, NRBCs in. . .

SUMM Reticulocytes are **red cells** at the **maturation** level just between NRBCs and mature RBCs. Reticulocytes provide a means of evaluating a patient's anemic state. Anemia usually occurs. . .

DETD . . . sample with a blood diluent which rapidly lyses RBC and preserves WBC, and to which has been added a suitable **nuclear stain** which will stain naked nuclei of the NRBC. Such a diluent is disclosed above. The diluent/sample mixture is then passed,. . .

DETD Vital **stains** (**nuclear stains** which **stain** only dead or damaged cells) that can be used in the present invention can be any vital stain with relatively. . .

DETD . . . with the triple trigger method, the reagent will additionally contain, or be combined with, a small concentration of a vital **nuclear stain** which effectively labels any NRBC which might be present in the peripheral blood. Preferably, for use with the herein referenced. . .

DETD . . . time be extended beyond 11 seconds. Additionally, in the preferred embodiment, the lyse contains a small concentration of a vital **nuclear stain** which effectively labels any nucleated red blood cells (NRBCs) which might be present in the peripheral blood. The lysis chemistry. . .

DETD Another method which uses extended incubation of the **nuclear stain** can also be used to measure reticulocytes by using both incubation probe 160 and aspiration probe 156 in a method. . .

L7 ANSWER 4 OF 9 USPATFULL on STN

ACCESSION NUMBER: 1999:43481 USPATFULL

TITLE: Method for performing automated analysis

INVENTOR(S): Gill, James E., Moutain View, CA, United States
Chupp, Vernon L., Los Altos, CA, United States
Hove, Luc Van, San Jose, CA, United States

PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5891734		19990406
APPLICATION INFO.:	US 1996-682334		19960717 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1995-482678, filed on 7 Jun 1995, now patented, Pat. No. US 5656499 which is a continuation-in-part of Ser. No. US 1994-283379, filed on 1 Aug 1994, now abandoned

DOCUMENT TYPE: Utility

FILE SEGMENT: Granted

PRIMARY EXAMINER: Le, Long V.

LEGAL REPRESENTATIVE: Bach, Mark C.

NUMBER OF CLAIMS: 8

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 115 Drawing Figure(s); 62 Drawing Page(s)

LINE COUNT: 4548

AB Provided are automated methods for distinguishing and differentiating cells in a whole blood sample. In one of the methods, a whole blood sample is provided. One or more tests to be performed on the whole blood sample is selected. The tests to be performed on the whole blood sample are correlated. A volume of the whole blood sample is aspirated into an automated instrument system which automatically performs conventional hematology analysis and fluorescent cytometry analysis on the whole blood sample. A first aliquot of the whole blood sample is dispensed into at least one sample receiving vessel. The first aliquot of the whole blood sample is mixed with a fluorescent reagent. The first aliquot of the whole blood sample mixed with fluorescent reagent is

diluted and transported through a flow transducer system. The flow transducer system detects multi-angle light scatter and fluorescence from the first aliquot of the whole blood sample mixed with fluorescent reagent and counts and differentiates platelets or platelet clumps or both in the sample. Detecting and differentiation data for the one or more tests performed on the whole blood sample are stored. Results of the one or more tests performed on the whole blood sample are reported in a quantitative manner if so requested. The instrument system automatically performs all method steps without physically separating cells from the whole blood sample or an aliquot of the sample and results of a conventional hematology analysis may be utilized in at least reporting of results of the fluorescent cytometry testing.

SUMM . . . relate to methods and devices for performing automated blood cell analysis by integrating "impedance," "light scattering," and "fluorescence" analysis and **flow cytometric** techniques. These embodiments also relate to a multipurpose reagent system and a method for rapid analysis of a whole blood. . .

SUMM The blood in an adult usually contains about 4.5 to 5 million RBCs or **erythrocytes** per cubic millimeter. **Mature** RBCs have no nuclei and are generally shaped as circular biconcave disks with a diameter of about 7.5 to 8. . .

SUMM Peripheral blood also contains **red cells** of earlier **maturation** levels which are important diagnostic indicators. Two of these are reticulocytes and **nucleated** red blood cells.

SUMM At the earliest stage of development the red cell consists mostly of nucleus, and is referred to as an **erythroblast**. As the **erythroblast** matures, the nucleus becomes smaller, anucleolate, and more nearly spherical. Subsequent maturity involves a complete loss of nucleus. The immature red cells that retain a nucleus are referred to as **nucleated** red blood cells (NRBCs). The NRBC count has been useful in patient monitoring under many disease states. However, NRBCs in. . .

SUMM Reticulocytes are **red cells** at the **maturation** level just between NRBCs and mature RBCs. Reticulocytes provide a means of evaluating a patient's anemic state. Anemia usually occurs. . .

DETD . . . sample with a blood diluent which rapidly lyses RBC and preserves WBC, and to which has been added a suitable **nuclear stain** which will stain naked nuclei of the NRBC. Such a diluent is disclosed above. The diluent/sample mixture is then passed,. . .

DETD Vital **stains** (**nuclear stains** which **stain** only dead or damaged cells) that can be used in the present invention can be any vital stain with relatively. . .

DETD . . . with the triple trigger method, the reagent will additionally contain, or be combined with, a small concentration of a vital **nuclear stain** which effectively labels any NRBC which might be present in the peripheral blood. Preferably, for use with the herein referenced. . .

DETD . . . time be extended beyond 11 seconds. Additionally, in the preferred embodiment, the lyse contains a small concentration of a vital **nuclear stain** which effectively labels any nucleated red blood cells (NRBCs) which might be present in the peripheral blood. The lysis chemistry. . .

DETD Another method which uses extended incubation of the **nuclear stain** can also be used to measure reticulocytes by using both incubation probe 160 and aspiration probe 156 in a method. . .

L7 ANSWER 5 OF 9 USPATFULL on STN

ACCESSION NUMBER: 97:70939 USPATFULL

TITLE: Method for performing automated hematology and cytometry analysis

INVENTOR(S): Chupp, Vernon L., Los Altos, CA, United States
Lobban, Peter E., Palo Alto, CA, United States
Kim, Young Ran, Sunnyvale, CA, United States

Larue, Roderick Walton, Sebastopol, CA, United States
Stuart, John Paul, Mt. View, CA, United States
PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5656499		19970812
APPLICATION INFO.:	US 1995-482678		19950607 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1994-283379, filed on 1 Aug 1994, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Le, Long V.
LEGAL REPRESENTATIVE: Bach, Mark C.
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 110 Drawing Figure(s); 61 Drawing Page(s)
LINE COUNT: 4357
AB A device for analyzing a whole blood sample is provided. The device comprises a conventionl hematology analyzer integrated with a fluorescence cyometry analyzer. A controller is provided for controlling the analyzers, obtaining and utilizing data from both and reporting a quantitative result. Methods are also provided for analyzing a whole blood sample. One such method comprises the steps of performing on a single instrument an analysis of impedance associated with the blood sample, an analysis of light scatter associated with the blood sample, and an analysis of fluorescence associated with the blood sample. Data is collected and utilized. A result is reported.

SUMM . . . relates to methods and devices for performing automated blood cell analysis by integrating "impedance," "light scattering," and "fluorescence" analysis and **flow cytometric** techniques. This invention also relates to a multipurpose reagent system and a method for rapid analysis of a whole blood. . .

SUMM The blood in an adult usually contains about 4.5 to 5 million RBCs or **erythrocytes** per cubic millimeter. **Mature** RBCs have no nuclei and are generally shaped as circular biconcave disks with a diameter of about 7.5 to 8. . .

SUMM Peripheral blood also contains **red cells** of earlier **maturation** levels which are important diagnostic indicators. Two of these are reticulocytes and **nucleated** red blood cells.

SUMM At the earliest stage of development the red cell consists mostly of nucleus, and is referred to as an **erythroblast**. As the **erythroblast** matures, the nucleus becomes smaller, anucleolate, and more nearly spherical. Subsequent maturity involves a complete loss of nucleus. The immature red cells that retain a nucleus are referred to as **nucleated** red blood cells (NRBCs). The NRBC count has been useful in patient monitoring under many disease states. However, NRBCs in. . .

SUMM Reticulocytes are **red cells** at the **maturation** level just between NRBCs and mature RBCs. Reticulocytes provide a means of evaluating a patient's anemic state. Anemia usually occurs. . .

DETD . . . sample with a blood diluent which rapidly lyses RBC and preserves WBC, and to which has been added a suitable **nuclear stain** which will stain naked nuclei of the NRBC. Such a diluent is disclosed above. The diluent/sample mixture is then passed,. . .

DETD Vital **stains** (**nuclear stains** which **stain** only dead or damaged cells) that can be used in the present invention can be any vital stain with relatively. . .

DETD . . . with the triple trigger method, the reagent will additionally contain, or be combined with, a small concentration of a vital **nuclear stain** which effectively labels any NRBC which might be present in the peripheral blood. Preferably, for use with the

herein referenced. . .

DETD . . . time be extended beyond 11 seconds. Additionally, in the preferred embodiment, the lyse contains a small concentration of a vital **nuclear stain** which effectively labels any nucleated red blood cells (NRBCs) which might be present in the peripheral blood. The lysis chemistry. . .

DETD Another method which uses extended incubation of the **nuclear stain** can also be used to measure reticulocytes by using both incubation probe 160 and aspiration probe 156 in a method. . .

L7 ANSWER 6 OF 9 USPATFULL on STN

ACCESSION NUMBER:	97:61562 USPATFULL
TITLE:	Method of using a multi-purpose beagent for subclassification of nucleated blood cells
INVENTOR(S):	Kim, Young Ran, Sunnyvale, CA, United States Kantor, Johanna, Palo Alto, CA, United States Gill, James E., Mountain View, CA, United States Luptovic, Sue E., San Jose, CA, United States
PATENT ASSIGNEE(S):	Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5648225		19970715
APPLICATION INFO.:	US 1994-296379		19940825 (8)
RELATED APPLN. INFO.:	Division of Ser. No. US 1993-23042, filed on 25 Feb 1993, now abandoned		

DOCUMENT TYPE:	Utility
FILE SEGMENT:	Granted
PRIMARY EXAMINER:	Hutzell, Paula K.
ASSISTANT EXAMINER:	Freed, Rachel Heather
LEGAL REPRESENTATIVE:	Poulos, Nicholas A.
NUMBER OF CLAIMS:	10
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	16 Drawing Figure(s); 16 Drawing Page(s)
LINE COUNT:	870

AB A multipurpose reagent system for rapid analysis of a whole blood sample allowing the determination of at least five classes of peripheral white blood cells, nucleated red blood cells, and lymphocyte immunophenotyping on automated hematology instrumentation. The multipurpose reagent system lyses red cells rapidly, while it concurrently fixes white cells and preserves surface antigens on lymphocytes. The multipurpose reagent system comprises from about 3 to 7 grams per liter of a non-quaternary ammonium salt, from about 0.04 to about 0.10 percent by volume of an aliphatic aldehyde with one to four carbons, from about 10 mM to about 20 mM of a non-phosphate buffer which is inert to the aliphatic aldehyde, and a sufficient amount of water to give a pH between 5.5 and 7.5 and an osmolality of between about 160 to about 310 mOsm per liter.

SUMM . . . rapidly lysing red cells and concurrently fixing white cells, useful for performing white cell differential analyses and quantitative analyses of **nucleated** red blood cells or lymphocyte subclassification using immunophenotyping techniques on an automated clinical hematology analyzer or **flow cytometer**.

SUMM The peripheral blood of a normal subject contains red blood cells, also known as **erythrocytes**, and five major classes of **mature** white cells, also known as leukocytes. There are at least five classes of leukocytes, known as neutrophils, eosinophils, monocytes, lymphocytes. . .

SUMM Recent advances in cellular immunology and **flow cytometry** are being utilized to identify and quantify lymphocyte subclasses such as helper T cells. Lymphocyte subclassification has become an important. . . lymphocytes with fluorochrome-labeled monoclonal antibodies directed to specific lymphocyte surface antigens; and (3) the analysis of lymphocyte-antibody reaction products using

flow cytometry. Currently, a great deal of effort is being directed towards the development of whole blood methods that bypass the need. . .

SUMM Generally speaking, the reagent systems available for use during the analysis of **nucleated** red blood cells (NRBC) are as yet unable to allow for the differentiation and counting of NRBC signals from red. . .

SUMM . . . for the present invention include a surface active agent such as saponin, an anticoagulant, an alkali salt of bicarbonate, a **nuclear stain**, or an antibody directed against specific cell surface antigens.

DRWD FIG. 3a shows a FACScan.TM. display printout of a normal blood sample, processed as described in Example 5 with a **nuclear stain** but without chicken erythrocyte nuclei (CEN);

DRWD . . . of a normal blood sample, supplemented with chicken erythrocyte nuclei, that was processed as described in Example 5 with a **nuclear stain**. An FL3 stained CEN population appears at the upper left hand corner;

DETD . . . automated hematology analyzers. In order to analyze the percentage of nucleated red cells present in a whole blood sample, a **nuclear stain**, e.g., ethidium homodimer, is added to the multipurpose reagent system before it is added to the blood sample In this embodiment, the **nuclear stain** is added to the reagent system in an amount from between about 0.05 mg % to about 0.15 mg %. . . lyses the red cells while simultaneously preserving the integrity of white cell membranes. In the multipurpose reagent system, the added **nuclear stain** reacts with the exposed nuclei of immature red cells, yet it is impenetrable to intact white cells. Since the only nuclear material available to interact with the **nuclear stain** is that from the nucleated red blood cells, the **stained nuclear** material is proportional to the nucleated erythrocyte fraction of the blood sample and can be quantitated on an automated electro-optical. . .

DETD . . . chicken erythrocyte nuclei (CEN processed as described in Example 5. The sample shown in FIG. 3a was processed with a **nuclear stain** but without CEN and the sample shown in FIG. 3b was processed in the presence of both a **nuclear stain** and CEN. The two dimensional dot plots on the left have plotted side scatter (SSC) versus forward scatter (FSC). The. . .

DETD . . . mixed with 950 microliters of the multipurpose reagent system of the present invention containing 0.1 mg % weight/volume of a **nuclear stain**, 0.5% weight/volume of ammonium chloride, 0.075% of volume of formaldehyde, 0.01% weight/volume of saponin, 0.01% weight/volume of potassium bicarbonate, and. . .

CLM What is claimed is:

. . . the multipurpose reagent system further comprises from about 0.05 mg % to about 0.15 mg % by weight volume of **nuclear stain**

.

. . . The method of claim 1, wherein the multipurpose reagent system further comprises about 0.1 mg % by weight volume of **nuclear stain**.

L7 ANSWER 7 OF 9 USPATFULL on STN

ACCESSION NUMBER: 97:45185 USPATFULL

TITLE: In vivo use of human bone marrow for investigation and production

INVENTOR(S): Namikawa, Reiko, Palo Alto, CA, United States
Kyoizumi, Seishi, Hiroshima, Japan
McCune, Joseph M., San Francisco, CA, United States
Kaneshima, Hideto, Palo Alto, CA, United States

PATENT ASSIGNEE(S): Systemix, Inc., Palo Alto, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5633426		19970527
APPLICATION INFO.:	US 1994-194717		19940210 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1992-904886, filed on 25 Jun 1992, now abandoned And Ser. No. US 1993-90571, filed on 12 Jul 1993, now abandoned which is a continuation of Ser. No. US 1990-599649, filed on 18 Oct 1990, now abandoned , said Ser. No. US -904886 which is a continuation of Ser. No. US 1990-529217, filed on 25 May 1990, now abandoned

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Chambers, Jasemine C.
LEGAL REPRESENTATIVE: Sherwood, Pamela J.Fish & Richardson P.C.
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1,7
LINE COUNT: 883

AB Chimeric immunocompromised hosts are provided, comprising human bone marrow of at least 4 weeks from the time of implantation. The bone marrow is found to assume the normal population of bone marrow except for erythrocytes. The bone marrow may be used to study the effect of various agents on the proliferation and differentiation of hematopoietic cells.

DETD The human origin of hematopoietic cells within the grafts was confirmed by **flow cytometry** with either MEM-43 (an antibody specific for a common antigen of human cells) or Ly5.1 (reactive with mouse pan-leukocyte antigen).. . .

DETD The characteristics of the hematopoietic cell populations in the bone marrow were analyzed by light scattering profiles using **flow cytometry**. Four distinctive clusters of hematopoietic cells, i.e., lymphoid (RI), blastoid (R2), myeloid (R3), and mature granulocyte (R4) populations were revealed. . .

DETD The cell surface phenotypes of the **nucleated** hematopoietic cells in the grafts were further analyzed with various antibodies specific for human lineage markers (Table 1). About 80%. . . were found to be similar to those of normal fetal bone marrow. The proportion of these four regions within the **nucleated** hematopoietic cells in the grafted marrow was compared to that of normal fetal bone marrow. The percentage of mature granulocytes (R4 region) in total **nucleated** cells was found to be significantly lower in the grafts (12.+-.7%) than in the normal fetal marrow samples (25.+-.5%). In. . .

DETD The level of human erythropoietic activity was analyzed with antibodies specific for human glycophorin A (GPA). **Flow cytometric** analysis of human glycophorin A (GPA) expression in bone marrow cells from the grafts was performed. The cell suspensions were. . . were fixed in 2.5% paraformaldehyde in PBS, and then incubated with PI at the final concentration of 1 .mu.g/ml to **stain nuclear** DNA.

DETD 0.5-3% of **nucleated** cells (PI.sup.+) from 4-12 week implants were found to express a high level of GPA. Expression of GPA was also detected in a small number of cells in the enucleated cell population (PI), indicating that final **maturation** to human **erythrocytes** was possible in the bone implants. Compared to the number of **nucleated** erythroid cells in normal fetal bone marrow (approximately 30% when counted on cytospin preparations), this population was small. A low. . . cytospin preparations. Thus, although the level of erythropoiesis was lower than normal, human erythroid precursors were able to differentiate into **mature erythrocytes** in the grafted bones.

L7 ANSWER 8 OF 9 USPATFULL on STN

ACCESSION NUMBER: 97:42793 USPATFULL
TITLE: Method for performing automated hematology and cytometry analysis
INVENTOR(S): Chupp, Vernon L., Los Altos, CA, United States
Lobban, Peter E., Palo Alto, CA, United States
Kim, Young R., Sunnyvale, CA, United States
Larue, Roderick W., Sebastopol, CA, United States
Stuart, John P., Mt. View, CA, United States
PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5631165		19970520
APPLICATION INFO.:	US 1995-488532		19950607 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1994-283379, filed on 1 Aug 1994, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Le, Long V.
LEGAL REPRESENTATIVE: Bach, Mark C.
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 111 Drawing Figure(s); 61 Drawing Page(s)
LINE COUNT: 4306

AB A device for analyzing a whole blood sample is provided. The device comprises a conventional hematology analyzer integrated with a fluorescence cytometry analyzer. A controller is provided for controlling the analyzers, obtaining and utilizing data from both and reporting a quantitative result. Methods are also provided for analyzing a whole blood sample. One such method comprises the steps of performing on a single instrument an analysis of impedance associated with the blood sample, an analysis of light scatter associated with the blood sample, and an analysis of fluorescence associated with the blood sample. Data is collected and utilized. A result is reported.

SUMM . . . relates to methods and devices for performing automated blood cell analysis by integrating "impedance," "light scattering," and "fluorescence" analysis and **flow cytometric** techniques. This invention also relates to a multipurpose reagent system and a method for rapid analysis of a whole blood. . .

SUMM The blood in an adult usually contains about 4.5 to 5 million RBCs or **erythrocytes** per cubic millimeter. **Mature** RBCs have no nuclei and are generally shaped as circular biconcave disks with a diameter of about 7.5 to 8. . .

SUMM Peripheral blood also contains **red cells** of earlier **maturation** levels which are important diagnostic indicators. Two of these are reticulocytes and **nucleated** red blood cells.

SUMM At the earliest stage of development the red cell consists mostly of nucleus, and is referred to as an **erythroblast**. As the **erythroblast** matures, the nucleus becomes smaller, anucleolate, and more nearly spherical. Subsequent maturity involves a complete loss of nucleus. The immature red cells that retain a nucleus are referred to as **nucleated** red blood cells (NRBCs). The NRBC count has been useful in patient monitoring under many disease states. However, NRBCs in. . .

SUMM Reticulocytes are **red cells** at the **maturation** level just between NRBCs and mature RBCs. Reticulocytes provide a means of evaluating a patient's anemic state. Anemia usually occurs. . .

DETD . . . sample with a blood diluent which rapidly lyses RBC and preserves WBC, and to which has been added a suitable **nuclear stain** which will stain naked nuclei of the NRBC. Such a diluent is disclosed above. The diluent/sample mixture is then passed,. . .

DETD Vital **stains** (**nuclear stains** which

stain only dead or damaged cells) that can be used in the present invention can be any vital stain with relatively. . .

DETD . . . with the triple trigger method, the reagent will additionally contain, or be combined with, a small concentration of a vital **nuclear stain** which effectively labels any NRBC which might be present in the peripheral blood. Preferably, for use with the herein referenced. . .

DETD . . . time be extended beyond 11 seconds. Additionally, in the preferred embodiment, the lyse contains a small concentration of a vital **nuclear stain** which effectively labels any nucleated red blood cells (NRBCs) which might be present in the peripheral blood. The lysis chemistry. . .

DETD Another method which uses extended incubation of the **nuclear stain** can also be used to measure reticulocytes by using both incubation probe 160 and aspiration probe 156 in a method. . .

L7 ANSWER 9 OF 9 USPATFULL on STN

ACCESSION NUMBER: 96:41135 USPATFULL

TITLE: Multipurpose reagent system for rapid lysis of whole blood

INVENTOR(S): Kim, Young R., Sunnyvale, CA, United States
Kantor, Johanna, Palo Alto, CA, United States
Gill, James E., Mountain View, CA, United States
Luptovic, Sue E., San Jose, CA, United States

PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5516695		19960514
APPLICATION INFO.:	US 1994-297662		19940829 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1993-23042, filed on 25 Feb 1993, now abandoned

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Wityshyn, Michael G.
ASSISTANT EXAMINER: Freed, Rachel Heather
LEGAL REPRESENTATIVE: Schmidt, Richard D.
NUMBER OF CLAIMS: 27
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 16 Drawing Figure(s); 16 Drawing Page(s)
LINE COUNT: 910
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A multipurpose reagent system for rapid analysis of a whole blood sample allowing the determination of at least five classes of peripheral white blood cells, nucleated red blood cells, and lymphocyte immunophenotyping on automated hematology instrumentation. The multipurpose reagent system lyses red cells rapidly, while it concurrently fixes white cells and preserves surface antigens on lymphocytes. The multipurpose reagent system comprises from about 3 to 7 grams per liter of a non-quaternary ammonium salt, from about 0.04 to about 0.10 percent by volume of an aliphatic aldehyde with one to four carbons, from about 10 mM to about 20 mM of a non-phosphate buffer which is inert to the aliphatic aldehyde, and a sufficient amount of water to give a pH between 5.5 and 7.5 and an osmolality of between about 160 to about 310 mOsm per liter.

SUMM . . . rapidly lysing red cells and concurrently fixing white cells, useful for performing white cell differential analyses and quantitative analyses of **nucleated** red blood cells or lymphocyte subclassification using immunophenotyping techniques on an automated clinical hematology analyzer or **flow cytometer**.

SUMM The peripheral blood of a normal subject contains red blood cells, also known as **erythrocytes**, and five major classes of **mature** white cells, also known as leukocytes. There are at least five classes of leukocytes, known as neutrophils, eosinophils,

monocytes, lymphocytes. . .

SUMM Recent advances in cellular immunology and **flow cytometry** are being utilized to identify and quantify lymphocyte subclasses such as helper T cells. Lymphocyte subclassification has become an important. . . lymphocytes with fluorochrome-labeled monoclonal antibodies directed to specific lymphocyte surface antigens; and (3) the analysis of lymphocyte-antibody reaction products using **flow cytometry**. Currently, a great deal of effort is being directed towards the development of whole blood methods that bypass the need. . .

SUMM Generally speaking, the reagent systems available for use during the analysis of **nucleated** red blood cells (NRBC) are as yet unable to allow for the differentiation and counting of NRBC signals from red. . .

SUMM . . . for the present invention include a surface active agent such as saponin, an anticoagulant, an alkali salt of bicarbonate, a **nuclear stain**, or an antibody directed against specific cell surface antigens.

DRWD FIG. 3a shows a FACScan.TM. display printout of a normal blood sample, processed as described in Example 5 with a **nuclear stain** but without chicken erythrocyte nuclei (CEN);

DRWD . . . of a normal blood sample, supplemented with chicken erythrocyte nuclei, that was processed as described in Example 5 with a **nuclear stain**. An FL3 stained CEN population appears at the upper left hand corner;

DETD . . . automated hematology analyzers. In order to analyze the percentage of nucleated red cells present in a whole blood sample, a **nuclear stain**, e.g., ethidium homodimer, is added to the multipurpose reagent system before it is added to the blood sample. In this embodiment, the **nuclear stain** is added to the reagent system in an amount from between about 0.05 mg % to about 0.15 mg %. . . lyses the red cells while simultaneously preserving the integrity of white cell membranes. In the multipurpose reagent system, the added **nuclear stain** reacts with the exposed nuclei of immature red cells, yet it is impenetrable to intact white cells. Since the only nuclear material available to interact with the **nuclear stain** is that from the nucleated red blood cells, the **stained nuclear** material is proportional to the nucleated erythrocyte fraction of the blood sample and can be quantitated on an automated electro-optical. . .

DETD . . . chicken erythrocyte nuclei (CEN) processed as described in Example 5. The sample shown in FIG. 3a was processed with a **nuclear stain** but without CEN and -the sample shown in FIG. 3b was processed in the presence of both a **nuclear stain** and CEN. The two dimensional dot plots on she left have plotted side scarlet (SSC) versus forward scatter (FSC). The. . .

DETD . . . mixed with 950 microliters of the multipurpose reagent system of the present invention containing 0.1 mg % weight/volume of a **nuclear stain**, 0.5% weight/volume of ammonium chloride, 0.075% of volume of formaldehyde, 0.01% weight/volume of saponin, 0.01% weight/volume of potassium bicarbonate, and. . .

CLM What is claimed is:

. . . as recited in claim 1, further comprising from about 0.05 mg % to about 0.15 mg% by weight volume of **nuclear stain**.

. . . 25. The multipurpose reagent system as recited in claim 1, further comprising about 0.1 mg % by weight volume of **nuclear stain**.

26. The multipurpose reagent system as recited in claim 17, further comprising 0.1 mg % of **nuclear stain**.

27. A diagnostic kit useful for the determination of nucleated erythrocytes comprising: the multipurpose reagent system as recited in

claim 17, and a solution of **nuclear stain**.

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=> d his

     (FILE 'HOME' ENTERED AT 15:46:21 ON 12 NOV 2003)

     FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, USPATFULL' ENTERED AT 15:46:37
     ON 12 NOV 2003
L1          4512 S (ERYTHROC? OR (RED CELL?)) (5A) MATUR?
L2           244 S L1 (6P) FLOW CYTOMET?
L3           486 S L1 (6P) ERYTHROBLAST?
L4           736 S L1 (6P) (ERYTHROBLAST? OR NUCLEATED)
L5            82 S L4 AND L2
L6            52 DUP REM L5 (30 DUPLICATES REMOVED)
L7             9 S L6 AND ((NUCLEOTIDE OR NUCLEAR) (2A) STAIN?)

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